17
2. A process for the preparation of a compound of the formula I,

B(1-30)-Arg-A(1-21) (I),

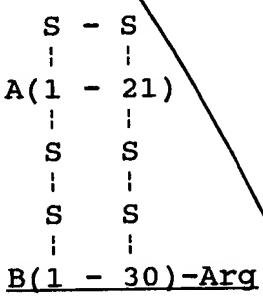
which comprises expressing a gene structure encoding for this compound in a bacterium and, if the gene structure also encodes a fusion protein, liberating the compound of the formula I from the fusion protein.

18
3. A DNA encoding for the compound of the formula I.

19
4. A gene structure or plasmid containing the DNA as claimed in claim 18.

5. A bacterium containing the gene structure or plasmid as claimed in claim 19.

6. A method for the preparation of a compound of the formula II



(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, using the compound of the formula I which comprises:

(a) expressing a DNA molecule encoding the compound of the formula I in a bacterium; and

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

(b) incubating the expressed compound of the formula I resulting from step (a) with trypsin under slightly acidic conditions at a pH of about 6.8 where phenol and other similar aromatics are not present.

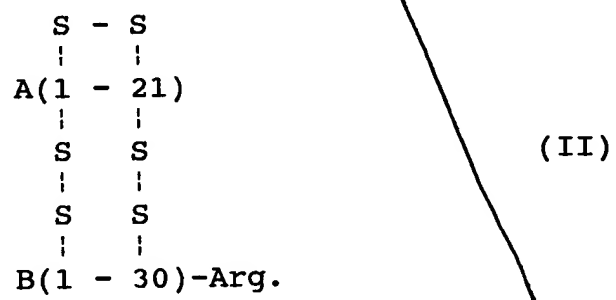
22. A method for the preparation of insulin using the compound of the formula I which comprises:

(a) expressing a DNA molecule enccding the compound of the formula I in a bacterium;

(b) incubating the expressed compound of the formula I resulting from step (a) with trypsin under slightly acidic conditions at a pH of about 6.8 where phenol and other similar aromatics are not present; and

(c) cleaving the resulting compound of the formula II with carboxypeptidase B.

23. A method as claimed in claim 22 wherein steps (b) and (c) are carried out in one vessel without having to isolate an intermediate compound of the formula II



(II)

24. A fusion protein which comprises the compound of the formula I,

B(1-30)-Arg-A(1-21) (I),

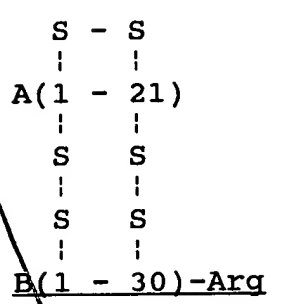
bonded via a bridging member

- Met - Ile - Glu - Gly - Arg -

to a peptide which stabilizes the fusion protein.

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N. W.
WASHINGTON, DC 20005
1-202-408-4000

25
~~10~~. A method for the preparation of a compound of the formula II



(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing a DNA molecule encoding the fusion protein of claim 24 ~~9~~ in a bacterium;

(b) cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide, thereby producing mini-proinsulin; and

(c) incubating the mini-proinsulin of step (b) with trypsin under slightly acidic conditions at a pH of about 6.8 where phenol and other similar aromatics are not present.

26
~~11~~. A method for the preparation of insulin which comprises:

(a) expressing a DNA molecule encoding the fusion protein of claim 24 ~~9~~ in a bacterium;

(b) cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide, thereby producing mini-proinsulin;

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

(c) incubating the mini-proinsulin of step (b) with trypsin under slightly acidic conditions at a pH of about 6.8 where phenol and other similar aromatics are not present; and

(d) cleaving the resulting compound of the formula II with carboxypeptidase B.

27 ~~12~~. A method as claimed in claim 26 ~~11~~ wherein steps (c) and (d) are carried out in one vessel without having to isolate an intermediate compound of the formula II

```
      S - S
      |   |
A(1 - 21)
      |   |
      S   S                    (II)
      |   |
      S   S
      |   |
B(1 - 30)-Arg.
```

28 ~~13~~. A compound of the formula I,

B(1-30)-Arg-A(1-21) (I),

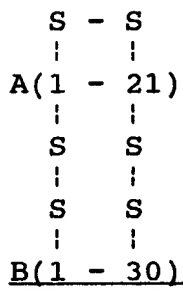
in which B(1-30) and A(1-21) denote the B and A chains of human insulin, which is formed by the process which comprises:

(a) expressing a DNA molecule encoding for the compound of the formula I in a bacterium; and

(b) when said compound of the formula I is part of a fusion protein, liberating the expressed compound of the formula I resulting from step (a) from the fusion protein.

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000

29 ~~14~~. A compound of the formula II



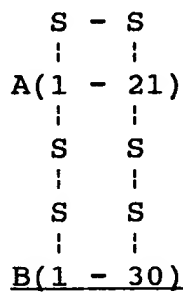
(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which is formed by the process which comprises:

(a) expressing a DNA molecule encoding for the compound of the formula I in a bacterium; and

(b) incubating the expressed compound of the formula I resulting from step (a) with trypsin under slightly acidic conditions at a pH of about 6.8 where phenol and other similar aromatics are not present.

30 ~~15~~. A compound of the formula II



(II)

in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which is formed by the process which comprises:

(a) expressing a DNA molecule encoding the fusion protein of claim ~~9~~ 24 in a bacterium;

LAW OFFICES
FINNEGAN, HENDERSON
FARABOW, GARRETT
& DUNNER
1300 I STREET, N.W.
WASHINGTON, DC 20005
1-202-408-4000